

## SUPPLEMENTARY MATERIAL

### **Structural analysis and antidiabetic activity study of three acidic-type polysaccharides from *Crepis crocea* (Lam.) Bab.**

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### **Abstract**

In this paper, we analyzed the structures of three acidic hetero-chain polysaccharides (CTP3-B, CTP3-C, and CTP3-D) fractionated from the herb *Crepis crocea* (Lam.) Bab. by a combination of ethanol precipitation, dialysis and gel permeation chromatography. Three polysaccharides were all highly branched polysaccharide. KK-Ay mice were chosen to determine the hypoglycemic effect of CTP3. The anti-diabetic activity of CTP3 was explored in detail from the aspects of body weight, daily dietary intake, blood glucose level and oral glucose tolerance test (OGTT). It was found that the body weight and daily food intake of the high dose group were significantly decreased compared with the diabetic control group. In addition, there was significant decrease in the levels of blood glucose in the middle and high dose group. These results suggest that CTP3 had a regulative role in blood glucose level. CTP3 may be useful in the treatment of type 2 diabetes mellitus.

Keywords: *Crepis crocea* (Lam.) Babc.; acidic hetero-chain polysaccharides; blood glucose levels; type 2 diabetics

## **Experimental**

### ***1. Chemical reagents and animals***

DEAE-Cellulose 52 was purchased from Whatman (UK). Sephadex G100 was purchased from Uppsala (Sweden). Dialysis bags (flat width 34 mm, molecular weight cut off 3500) were bought from BioDee biotechnology co. Ltd (Beijing, China). Dextran T-series standards (Mw: 4600, 7100, 21400, 41100, 133800) and serial standard monosaccharides (D-mannose, L-rhamnose, D-galactose, L-arabinose, D-glucose, D-galacturonic acid and D-glucuronic acid) were purchased from National Institutes for Drugs and Biological Products (Beijing, China). 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride were purchased from Kayon (USA). All other chemicals and reagents were analytical grade. The KK-Ay mice were used as a diabetic model, while C57BL/6 mice were employed as the non-diabetic control. All mice were purchased from the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences (license No. SCXK (Beijing)-2009-0004, Beijing, China). All animal studies were approved by the Ethical Committee of Shanxi Institute of Traditional Chinese Medicine (SZYLY2009KY-0201) and were performed according to the guidelines of the Laboratory Animal Management Regulations in China and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, revised 2011).

## **2. Preparation of Polysaccharides**

The *Crepis crocea* (Lam.) Bab. plants were collected in Shanyin County, Shanxi Province, China, and authenticated by Professor Tian'ai Gao, Shanxi Province Institute for Food and Drug Control. The dry samples were extracted three times (60 min) with distilled water at 95 °C. All water-extracts were combined, filtrated, concentrated under a reduced pressure, and then precipitated by adding 95% ethanol to a final concentration of 40% at room temperature for 12 h. The supernatant was collected, mixed with ethanol to a 60% concentration, and then centrifuged to get the crude polysaccharide (CP). Subsequently, the CP was deproteinated and depigmented with D900 macroporous resin. The eluent was combined, and the dialysis bag with a molecular weight cut-off of 3500 Da was used to remove small molecule substances against distilled water for 48 h. The retained fraction was precipitated with ethanol to an 80% final concentration. The resulting precipitate was collected by centrifugation, and dried in a vacuum freeze drier until its weight was constant. Finally, the aqueous polysaccharide fraction of *Crepis crocea* (Lam.) Bab. (CTP3) was obtained.

## **3. Purification of CTP3**

The CTP3 sample (700 mg) was dissolved in deionized water (15 mL) and centrifuged. Then the solution was applied to a DEAE-Cellulose 52 column (2.5 cm × 300 mm) equilibrated previously with deionized water for further

purification. The column was step-wise eluted with gradient NaCl aqueous solutions (0-0.5 mol/L). The polysaccharide content in each fraction was monitored by UV spectrophotometer. Consequently, three main fractions eluted out of Sephadex G100 gel column with distilled water at a flow rate of 1.0 mL/min were collected, concentrated, dialyzed and lyophilized, designated as CTP3-B, CTP3-C, and CTP3-D for the following studies.

#### ***4. Analysis of homogeneity and molecular weight***

The polysaccharides (10 mg) were dissolved in 0.71% sodium sulfate solution (1 mL), and then the homogeneity and the molecular weight distribution were determined by high performance gel permeation chromatography (HPGPC), which was performed on a Shimadzu LC-10A HPLC system fitted with one TSK gel G4000PW<sub>XL</sub> column (7.8 mm × 30.0 cm, Tosoh, Japan), and a RID-10A refractive index detector (Shimadzu, Japan). The column was calibrated by Dextran T-series standards (MW: 4600、7100、21400、41100、133800). The mobile phase was 0.71% Na<sub>2</sub>SO<sub>4</sub>, and the flow rate was 0.5 mL/min at 35 °C.

#### ***5. Analysis of glycosyl composition***

The glycosyl compositions of CTP3-B, CTP3-C, and CTP3-D were determined according to the procedures reported previously with pre-column derivatization high performance liquid chromatography (HPLC) and UV detector. Briefly, polysaccharide (5 mg) was hydrolyzed with 2 mL of 2 mol/L

trifluoroacetic acid (TFA) at 100 °C for 6 h in a sealed tube. After removing the residual TFA under reduced pressure below 40 °C, the hydrolyzed sample was mixed with 3 mL methanol. The above steps were repeated for four times to remove TFA completely, and the sample was then put into a vacuum dryer at 40 °C overnight. The dry hydrolysate was dissolved in 1 mL of distilled water, and then 100 µL of them was derivatized with 100 µL of 0.5 mol/L PMP methanol solution and 0.3 mol/L sodium hydroxide solution in water bath at 70 °C for 1 h. After the derivatization was cooled, 100 µL of 0.3 mol/L hydrochloric acid solution was added to neutralize the residual alkali solution. Subsequently, the above solution was dissolved into 1 mL of distilled water, and then extracted using the same amount of chloroform for 3 times. The water phase was filtered through 0.45 µm nylon membrane for HPLC analysis.

The derivatives were analyzed by an Agilent HPLC system equipped with a Sino-Chrom ODS-BP (5 µm 4.6 mm × 250 mm) and an XDB-UV detector. The mobile phase was a mixture with a flow rate of 1.0 mL/min by gradient elution and the compositions were as follows: solvent A was composed of acetonitrile and 0.05 mol/L phosphate buffer solution (KH<sub>2</sub>PO<sub>4</sub>-NaOH, pH 6.8) with the ratio of 15% (V/V), and solvent B was composed of acetonitrile and 0.05 mol/L phosphate buffer solution (KH<sub>2</sub>PO<sub>4</sub>-NaOH, pH 6.9) with the ratio of 40% (V/V). The time gradient of solvent A was 0→14→35 min and the concentration gradient was 0→20%→40%. Several standard monosaccharide samples were used as control under the same conditions.

## ***6. Glycosyl linkage analysis***

The glycosyl-linkage composition of CTP3-B, CTP3-C, and CTP3-D were performed by using the reported procedure with slight modification. In brief, 20 mg of dry polysaccharides sample was dissolved in 6 mL of DMSO. When the sample was completely dissolved, 240 mg NaOH mixed with 6 mL DMSO was added. The mixture was then stirred for 1 h at 25 °C. After a majority of NaOH dissolved, 3.6 mL of methyl iodide was added for polysaccharide methylation. The sample was shaken on the vortex mixer for 7 min. After that, the methylation was terminated by adding 6 mL H<sub>2</sub>O. The sample was extracted using the same amount of chloroform. The organic phase was concentrated under reduced pressure condition, and the obtained sample was analyzed by the infrared spectrum detection. The above operations were repeated until the methylation was completely analyzed. The methylated sample was dissolved in a 1 mL of 85% formic acid solution and was plugged and depolymerized at 100 °C for 4 h. Methanol was then added, and the solution was evaporated to dryness at 40 °C. Then 2 mL of 2 mol/L TFA was added to depolymerized sample, which was hydrolyzed at 100 °C for 6 h. Ethanol was then added, and the solution was evaporated to dryness at 40 °C. 70 mg NaBH<sub>4</sub> was added to reduce the uronic acid. After incubation at 25 °C for 12 h, acetic acid was added to terminate the reduction. The partially methylated residues were dried under low pressure and then acetylated by 0.5 mL of acetic anhydride and 0.5 mL of pyridine. The reaction was kept at 100 °C for 2 h and then dried. The

acetylated derivative was dissolved in chloroform, filtered through 0.22  $\mu\text{m}$  nylon membrane for GC-MS assay (Agilent 5975C GC-MS) with HP-5 quartz capillary column. The temperature program was set as follows: the initial temperature of the column was 145  $^{\circ}\text{C}$ , then increased to 165  $^{\circ}\text{C}$  at 5  $^{\circ}\text{C}/\text{min}$  and held for 5 min at 165  $^{\circ}\text{C}$ , then increased to 190  $^{\circ}\text{C}$  at 5  $^{\circ}\text{C}/\text{min}$  and held for 5 min at 190  $^{\circ}\text{C}$ , and finally increased to 245  $^{\circ}\text{C}$  at 10  $^{\circ}\text{C}/\text{min}$  and held for 5 min at 245  $^{\circ}\text{C}$ . The injection temperature was 230  $^{\circ}\text{C}$ . The ion source of the mass spectrometer was set at 230  $^{\circ}\text{C}$ .

## **7. NMR analysis**

All spectra including  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, DEPT-135,  $^1\text{H}$ - $^1\text{H}$  COSY, HMBC and HMQC were recorded on Bruker DRX-600 spectrometer (Bruker, Rheinstetten, Germany) using TMS as internal standard and  $\text{D}_2\text{O}$  as the solvents at 25  $^{\circ}\text{C}$ .

## **8. Anti-hyperglycemic activity study**

Nine weeks old KK-Ay mice ( $36 \pm 2$  g) were used to establish the type 2 DM model. C57BL/6 mice of the same age ( $26 \pm 1$  g) fed with standard diet were taken as a non-diabetic control. For both trials, the ratio of female to male was selected to be 50:50. All animals were kept at single metabolic cages and maintained under the conditions of temperature ( $23 \pm 1$   $^{\circ}\text{C}$ ), humidity ( $55 \pm 5\%$ ) and 12 h photoperiod. The mice were allowed free access to tap water and food (standard rod-like diet). All animal experiments were carried out in



accordance with the Principles of Laboratory Animal Care.

The fasting blood glucose values of KK-Ay mice were measured after one week's adaptive feeding. Sixty KK-Ay mice, whose blood glucose values reached 15 mmol/L or higher, were selected for the following study. The mice were then randomly assigned into 5 groups with 12 mice in each: the diabetic model group, the Acarbose treatment group (100 mg/kg/d), as well as CTP3 low (40 mg/kg/d), medium (80 mg/kg/d) and high dose groups (160 mg/kg/d). The mice of different CTP3 dose groups were daily administrated with different doses of CTP3 by gavage. In addition, 12 C57BL/6 mice fed with normal diet were taken as the non-diabetic control. The mice in diabetic model group and the mice in the non-diabetic control group were daily given equal amount of distilled water by gavage. During administration, body weight and blood glucose levels were measured weekly. Each mouse was fed with 30 g diet in the metabolic cage at 9:00 a.m., and the remaining diet was weighed at 9:00 a.m. next morning to calculate the feeding quantity. After 4 weeks' administration, blood samples were drawn from the tail vein of mice and blood glucose levels were measured by a glucose analyzer (ACCU-CHEK Active, Roche, Shanghai, China). On the morning of OGTT, fasting animals received an oral gavage of glucose (2 g/kg) after the baseline blood was drawn. Then blood glucose levels were measured at 0, 30, 60 and 120 min after glucose administration.

## ***9. Statistical analysis***

Data were expressed as mean  $\pm$  SD (standard deviation) for 12 animals in each group. Statistically significant differences between the two groups were ascertained by means of Student's t-test. In all cases probability values of  $p < 0.05$  were taken as statistically significant.

**Table S1.** The average molecular weights (Mw) and monosaccharide compositions of CTP3-B, CTP3-C, and CTP3-D

Fraction	Mw (kDa)	Monosaccharide composition (molar ratio)						
		Gal	Ara	Glc	Rha	Man	GalA	GlcA
CTP3-B	74.7	121.52	77.31	18.87	3.73	8.09	1.00	1.09
CTP3-C	69.6	74.46	55.31	19.88	7.34	2.51	3.90	1.00
CTP3-D	67.3	119.33	74.33	26.78	14.98	3.08	5.90	1.00

**Table S2.** GC-MS results of methylated products of fractions of CTP-3

Fraction	Methylated product	Mass fragments (m/z)	Linkage type
<b>CTP3-B</b>	2,3,6-tri-O-methyl-D-Glucose	43,58,71,87,101,117,129,145,161	→4)-D-Glc-(1→
	3,4,6-tri-O-methyl-O-glucontride	43,58,71,87,101,117,129,145	→2)-D-Glc-(1→
	2,3,4,6-tetra-O-methyl-d-glucitol	43,73,85,115,145,187	D-Glc-(1→
	Methyl-.beta.-l-rhamnoside	43,58,87,117,129,159,201,233	β-L-Rha(1→
	2,4-di-O-methyl-Mannitol	43,58,87,101,117,129,143,159,189,233,	→3,6)-Man-(1→
	1,4,5-Tri-O-acetyl-2,3-l-O-methylarabin	43,58,71,87,101,117,129,161	→5)-α-L-Ara-(1
	2,4-di-O-methyl-6-deoxy-D-galactitol	43,85,117,129,141,159	→3,6)-D-Gal-(1
	2,3,4-tri-O-methyl-D-Galactitol	43,71,87,101,117,129,143,161,173,233	→6)-D-Gal-(1→
<b>CTP3-C</b>	Methyl-.beta.-l-rhamnoside	43,58,87,117,129,159,201,233	β-L-Rha(1→
	1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-g	71,87,101,117,129,145,161,205	α-D-Glc-(1→
	2,3,6-tri-O-methyl-D-Glucose	43,58,71,87,101,117,129,145,161	→4)-α-D-Glc-(1
	2,3,4-tri-O-methyl-D-Glucose	43,58,71,87,101,117,129,145,161,173,	→6)-α-Glc-(1→
	beta.-d-Arabinopyranoside,methyl	43,86,101,128,157,170	β-D-Ara-(1→
	1,4,5-Tri-O-acetyl-2,3-l-O-methylarabin	43,58,71,87,101,117,129,161,189	→5)-α-L-Ara-(1
	2,5,6-Tri-O-acetyi-3,4-di-O-methyl-D-m	85,117,129,141,159,187	→2,6)-D-Man-(1
	2,4-di-O-methyl-6-deoxy-D-galactitol	43,117,129,141,187,201	→3,6)-D-Gal-(1
<b>CTP3-D</b>	2,3,4,6-tetra-O-acetyl-l-d-Galactopyrano	43,85,115,187,217,289	D-Gal-(1→
	2,3,6-tri-O-methyl-D-Glucose	43,58,71,87,101,117,129,145,161	→4)-D-Glc-(1→
	2,3,4-tri-methyl-D-Glucose	58,71,101,117,129,161,173,189	→6)-D-Glc-(1→
	2,3,4,6-tetra-O-methyl-d-glucitol	43,71,87,101,117,129,145,161,205	D-Glc-(1→
	2,4-di-O-methyl-Mannitol	43,58,87,101,117,129,143,189,233,245	→3,6)-D-Man-(1
	Methyl-.beta.-l-rhamnoside	43,58,87,117,129,159,201,233	β-L-Rha(1→
	1,4,5-Tri-O-acetyl-2,3-l-O-methylarabin	43,58,71,87,101,117,129,161	→5)-α-L-Ara-(1
	2,4-di-O-methyl-6-deoxy-D-galactitol	43,58,71,87,101,117,129,143,161,189,2	→3,6)-D-Gal-(1
<b>CTP3-D</b>	2,3,4-tri-O-methyl-D-Galactitol	43,58,71,87,101,117,129143,161,189,2	→6)-D-Gal-(1→

**Table S3.**  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts of CTP-3s recorded in  $\text{D}_2\text{O}$  at 25 °C

Fraction	Sugar residue	Chemical shifts (ppm)						References
		C-1/H-1	C-2/H-2	C-3/H-3	C-4/H-4	C-5/H-5	C-6/H-6	
CTP3-B	(A) $\rightarrow 3,6$ )- $\beta$ -D-Gal(1 $\rightarrow$	103.69/4.49	70.58/3.49	82.10/3.52	69.67/3.92	73.35/3.50	69.48/3.86,4.01	Gutiérrez et al. 2005; Wu and Wang 2008; Ali et al. 2011; Jahanbin et al. 2011; Khramova et al. 2011
	(B) $\rightarrow 6$ )- $\beta$ -D-Gal(1 $\rightarrow$	103.26/4.46	70.58/3.49	72.50/3.51	69.67/3.92	73.35/3.50	69.48/3.86,4.01	
	(C) $\alpha$ -D-Glc-(1 $\rightarrow$	99.67/4.95	72.59/3.50	73.36/3.98	72.91/3.31	71.81/3.50	61.20/3.80,3.63	Santos-Neves et al. 2008; Wu and Wang 2008; Ali et al. 2011; Jahanbin et al. 2011
	(D) $\rightarrow 2$ )- $\alpha$ -D-Glc-(1 $\rightarrow$	102.39/4.95	82.55/3.68	73.36/3.98	72.91/3.31	71.81/3.50	61.20/3.63,3.80	
	(E) $\rightarrow 4$ )- $\alpha$ -D-Glc-(1 $\rightarrow$	102.28/4.95	72.59/3.50	73.36/3.98	78.89/4.08	71.81/3.50	61.20/3.80,3.63	De Pinto G et al. 1998; Gutiérrez et al. 2005
	(F) $\beta$ -L-Rha(1 $\rightarrow$	101.39/5.04	74.90/3.63	72.78/3.31	74.90/3.69	71.67/3.78	16.64/1.25	
	(G) $\rightarrow 3,6$ )- $\alpha$ -D-Man-(1 $\rightarrow$	103.09/4.94	72.36/3.61	81.27/4.03	69.76/3.61	74.00/3.93	66.80/3.80,3.83	Chen et al. 2012
	(H) $\rightarrow 5$ )- $\alpha$ -L -Ara-(1 $\rightarrow$	109.24/5.20	79.8/4.20	75.80/3.64	81.06/3.87	68.50/3.77		Wu and Wang 2008; Jahanbin et al. 2011; Khramova et al. 2011; Cordeiro et al. 2012
	(I) $\rightarrow 4$ )- $\beta$ -D-GalA-(1 $\rightarrow$	104.45/4.55	76.53/4.00	77.79/3.91	83.82/4.01	76.51/3.74	174.96/—	De Pinto GL et al. 1998; Khramova et al. 2011; Cordeiro et al. 2012
	(J) $\alpha$ -D-GlcA-(1 $\rightarrow$	108.76/5.03	76.51/3.94	73.19/3.72	71.44/3.85	72.32/3.61	175.87/—	De Pinto GL et al. 1998; Gutiérrez et al. 2005
CTP3-C	(A) $\beta$ -D-Gal(1 $\rightarrow$	103.30/4.42	70.87/3.49	72.42/3.58	68.85/3.90	74.98/3.49	62.75/3.75,3.70	Gutiérrez et al. 2005; Wu and Wang 2008; Ali et al. 2011; Jahanbin et al. 2011; hramova et al. 2011
	(B) $\rightarrow 3,6$ )- $\beta$ -D-Gal(1 $\rightarrow$	103.00/4.49	70.82/3.48	81.25/3.49	68.85/3.91	74.98/3.49	69.10/3.84,3.94	
	(C) $\beta$ -L-Rha(1 $\rightarrow$	101.52/3.57	73.87/3.55	72.17/3.27	73.71/3.56	70.00/3.71	16.70/1.25	De Pinto G et al. 1998; Gutiérrez et al. 2005
	(D) $\alpha$ -D-Glc-(1 $\rightarrow$	101.24/4.99	72.42/3.58	74.02/3.95	72.85/3.33	71.67/3.52	61.09/3.78,3.63	Santos-Neves et al. 2008; Wu and Wang 2008;
	(E) $\rightarrow 6$ )- $\alpha$ -Glc-(1 $\rightarrow$	101.24/4.99	72.42/3.58	74.02/3.95	72.85/3.33	71.67/3.52	68.50/3.91,4.08	

	(F) $\rightarrow 4$ )- $\alpha$ -D-Glc-(1 $\rightarrow$	101.24/4.99	72.42/3.58	74.02/3.95	79.07/4.08	71.67/3.52	61.09/3.78,3.63	Ali et al. 2011; Jahanbin et al. 2011
	(G) $\rightarrow 2,6$ )- $\alpha$ -D-Man-(1 $\rightarrow$	101.47/5.19	81.24/4.12	70.73/3.83	69.73/3.60	73.44/3.85	66.80/3.73,3.79	Chen et al. 2012
	(H) $\alpha$ -L-Ara-(1 $\rightarrow$	107.31/5.02	83.91/4.09	72.36/3.61	79.72/3.88	61.56/3.79,3.86		Wu and Wang 2008; Jahanbin et al. 2011; Khramova et al. 2011; Cordeiro et al. 2012
	(I) $\rightarrow 5$ )- $\alpha$ -L-Ara-(1 $\rightarrow$	109.29/5.20	79.14/4.26	76.77/3.67	79.94/3.72	69.72/3.73,3.62		
	(J) $\rightarrow 4$ )- $\beta$ -D-GalA(1 $\rightarrow$	104.50/4.58	76.63/3.98	77.85/3.90	83.62/3.99	76.23/3.67	174.93/ -	De Pinto GL et al. 1998; Khramova et al. 2011; Cordeiro et al. 2012
	(K) $\alpha$ -D-GlcA-(1 $\rightarrow$	107.43/5.04	75.64/3.90	73.66/3.66	70.81/3.85	73.66/3.66	175.86/ -	De Pinto GL et al. 1998; Gutiérrez et al. 2005
<b>CTP3-D</b>	(A) $\rightarrow 3,6$ )- $\beta$ -D-Gal-(1 $\rightarrow$	103.45/4.61	70.63/3.48	81.17/3.45	69.69/3.71	74.83/3.49	68.52/4.17,3.88	Gutiérrez et al. 2005;
	(B) $\rightarrow 6$ )- $\beta$ -D-Gal-(1 $\rightarrow$	103.51/4.46	70.63/3.48	72.11/3.59	69.69/3.71	74.89/3.50	68.52/4.17,3.88	Wu and Wang 2008; Ali et al. 2011; Jahanbin et al. 2011; Khramova et al. 2011
	(C) $\alpha$ -D-Glc-(1 $\rightarrow$	100.28/4.98	73.40/3.65	73.92/3.96	72.84/3.30	71.06/3.60	61.30/3.76,3.62	Santos-Neves et al. 2008; Wu and Wang 2008; Ali et al. 2011; Jahanbin et al. 2011
	(D) $\rightarrow 4$ )- $\alpha$ -D-Glc-(1 $\rightarrow$	100.28/4.98	73.40/3.65	73.92/3.96	79.23/3.83	71.06/3.55	61.30/3.76,3.62	
	(E) $\rightarrow 6$ )- $\alpha$ -Glc-(1 $\rightarrow$	100.28/4.98	73.40/3.65	73.92/3.96	72.84/3.29	71.06/3.42	69.80/4.05,3.87	
	(F) $\beta$ -L-Rha-(1 $\rightarrow$	101.27/5.02	72.41/3.46	72.19/3.19	72.44/3.46	69.60/3.68	16.90/1.16	De Pinto G et al. 1998; Gutiérrez et al. 2005
	(G) $\rightarrow 3,6$ )- $\alpha$ -D-Man-(1 $\rightarrow$	102.60/5.00	72.49/3.70	80.85/4.05	69.81/3.59	73.29/3.82	66.67/3.73,3.79	Chen et al. 2012
	(H) $\rightarrow 5$ )- $\alpha$ -L -Ara-(1 $\rightarrow$	109.17/5.18	81.28/4.13	75.70/3.70	79.93/3.87	69.30/3.70,3.87		Wu and Wang 2008; Jahanbin et al. 2011; Khramova et al. 2011; Cordeiro et al. 2012
	(I) $\rightarrow 4$ ) $\beta$ -D-GalA-(1 $\rightarrow$	103.61/4.60	76.62/4.05	77.40/3.92	83.40/4.01	75.90/3.73	174.35/—	De Pinto GL et al. 1998; Khramova et al. 2011; Cordeiro et al. 2012
	(J) $\alpha$ -D-GlcA-(1 $\rightarrow$	108.29/5.02	75.91/3.99	73.70/3.71	70.60/3.85	72.60/3.61	174.67/—	De Pinto GL et al. 1998; Gutiérrez et al. 2005

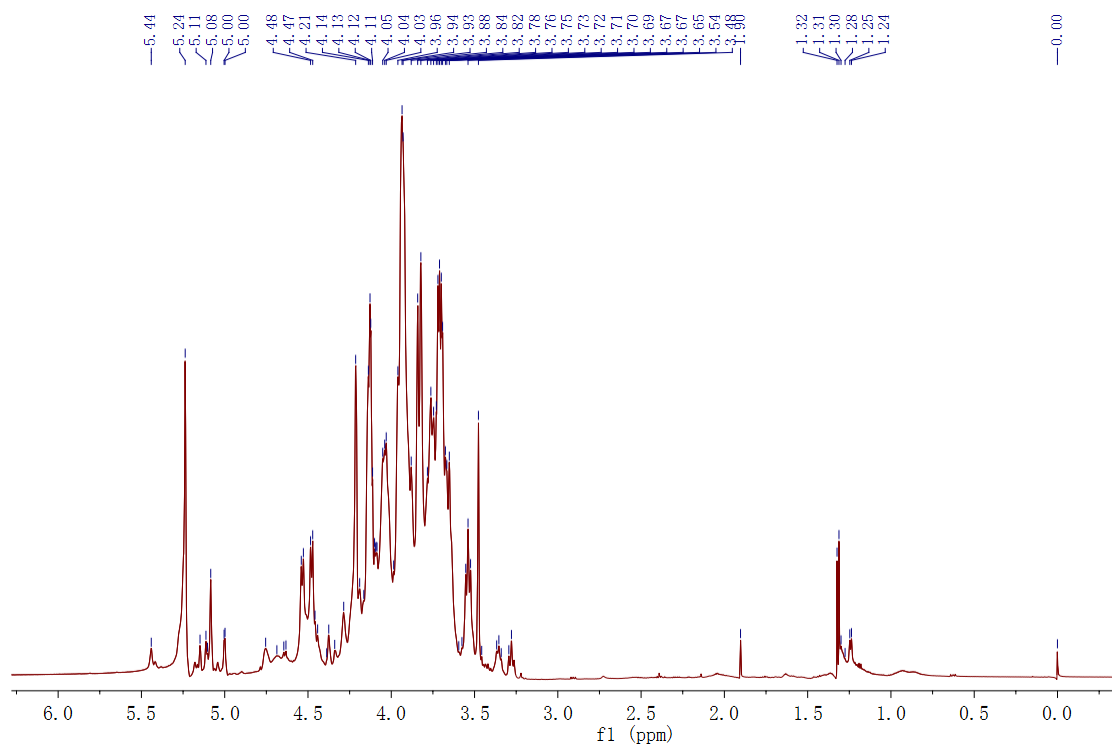


Figure S1 The  $^1\text{H}$  NMR spectra of CTP3-B

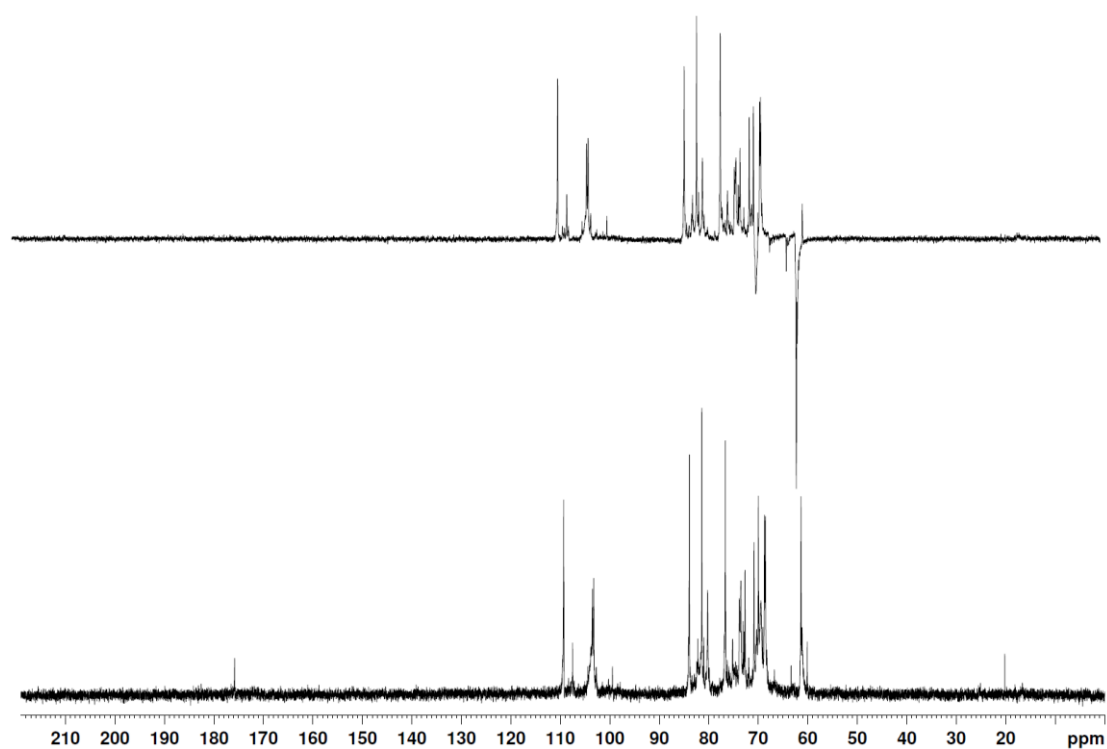


Figure S2 The  $^{13}\text{C}$  NMR spectra of CTP3-B



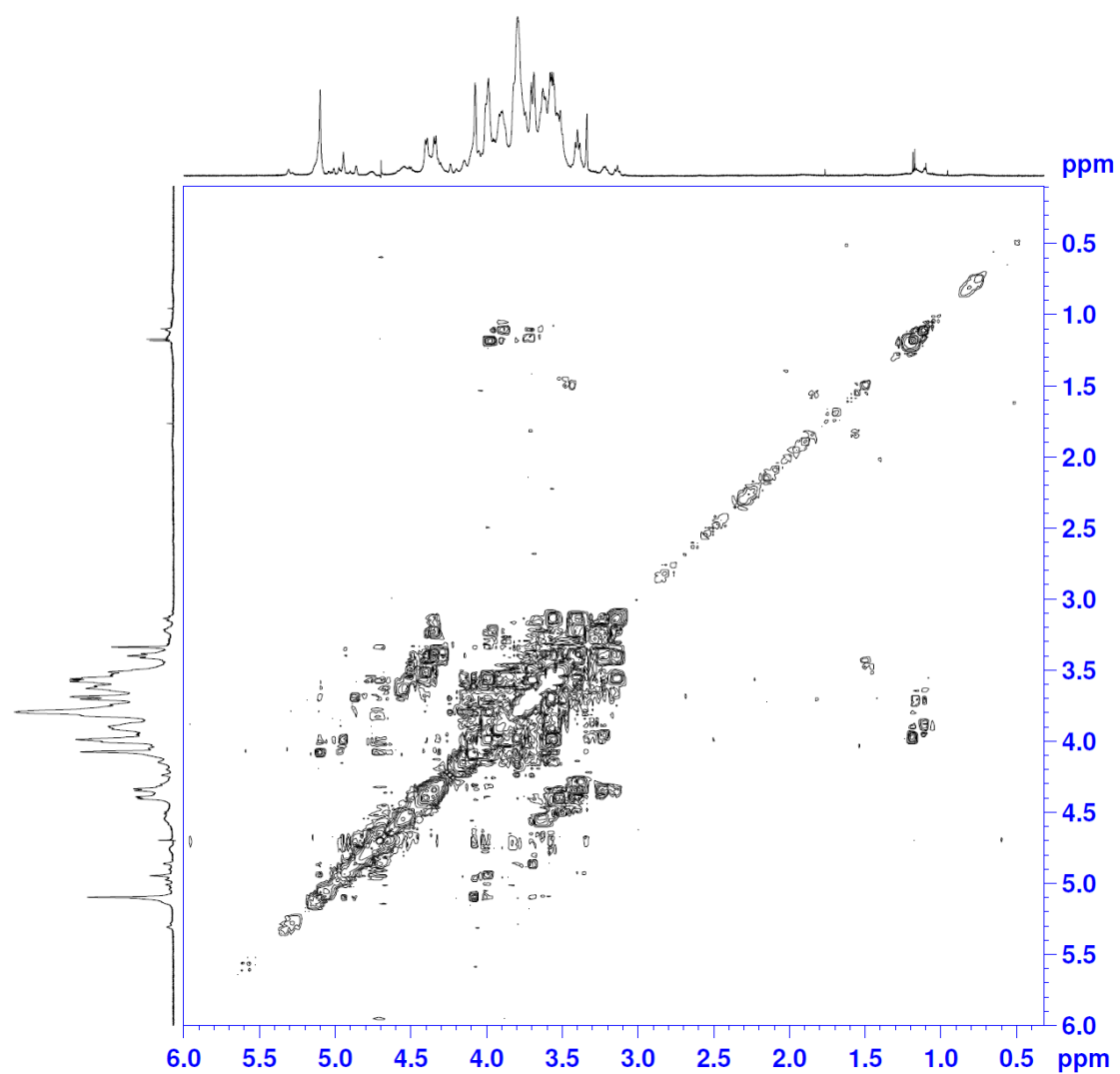


Figure S3 The COSY spectrum of CTP3-B

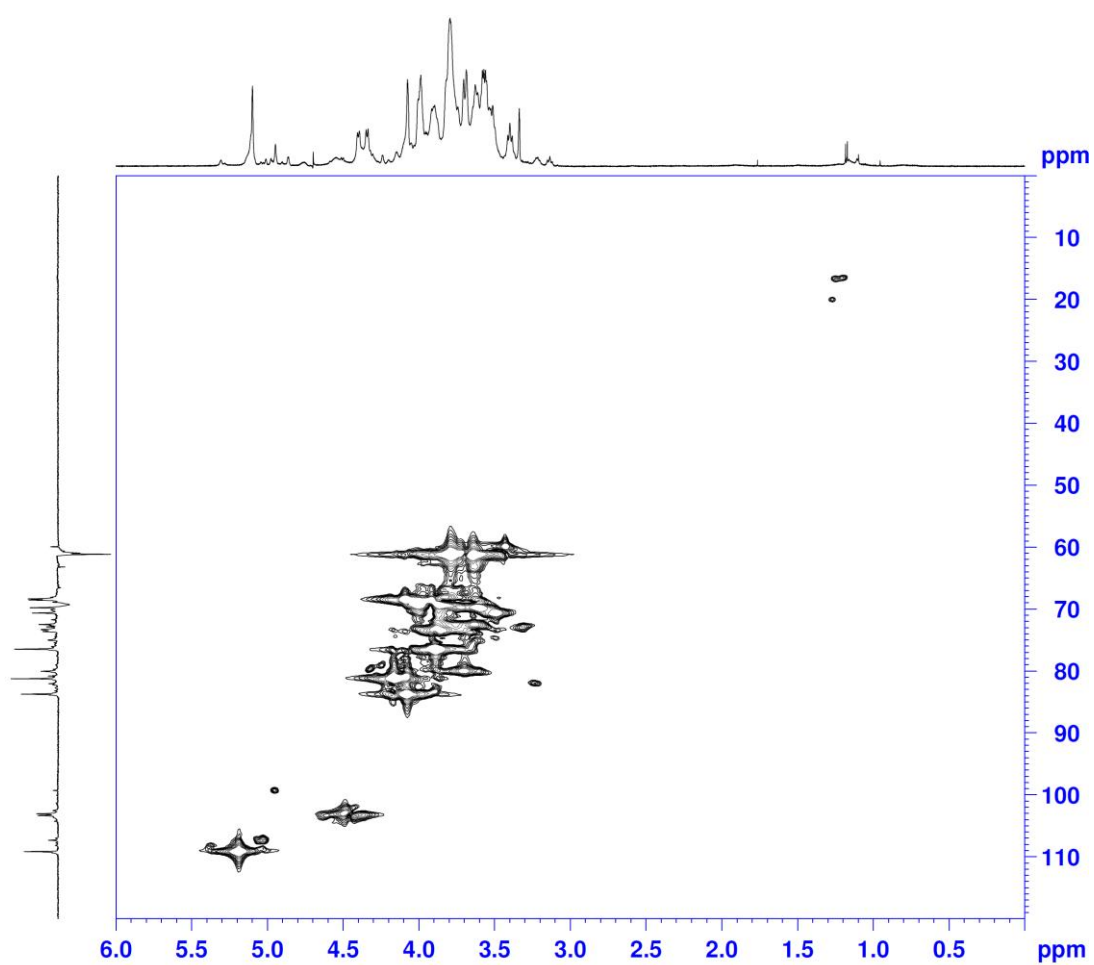


Figure S4 The HMQC spectrum of CTP3-B

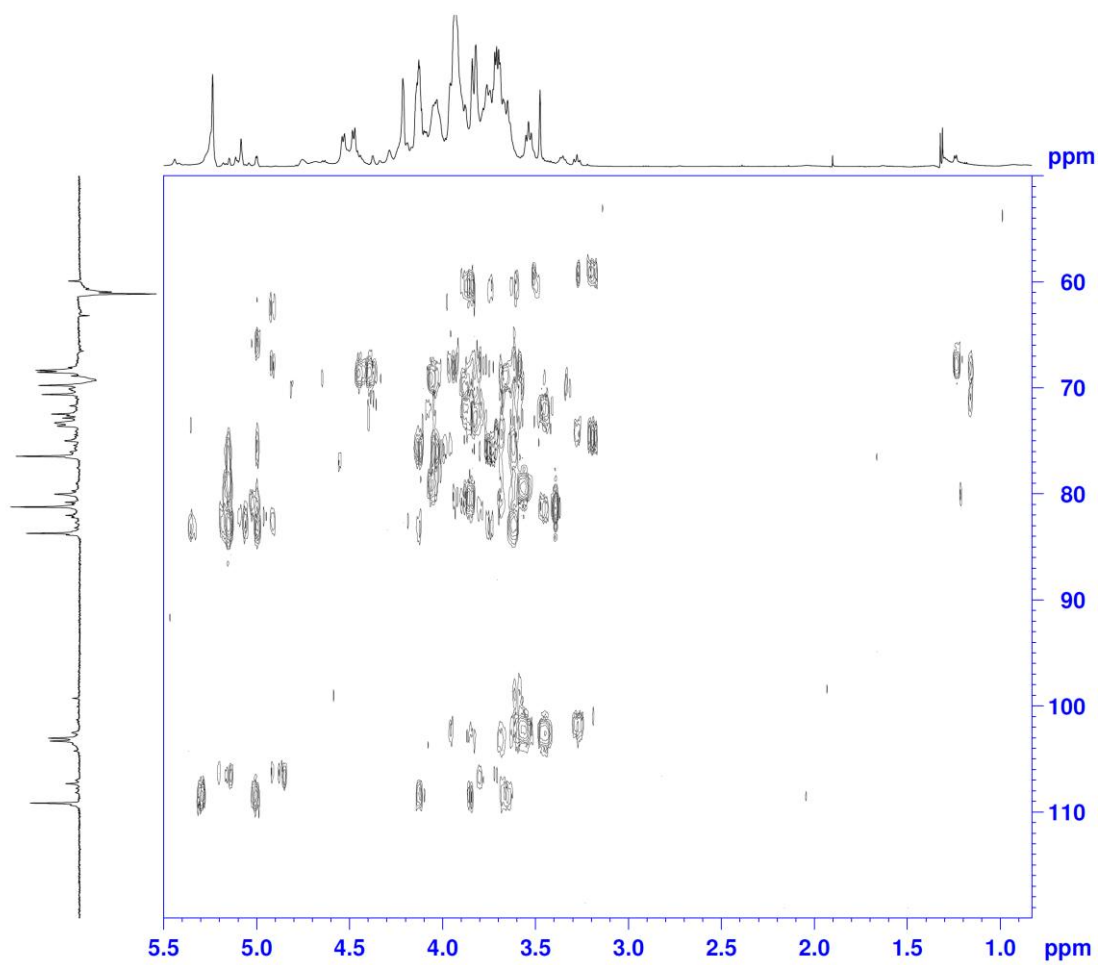


Figure S5 The HMBC spectrum of CTP3-B

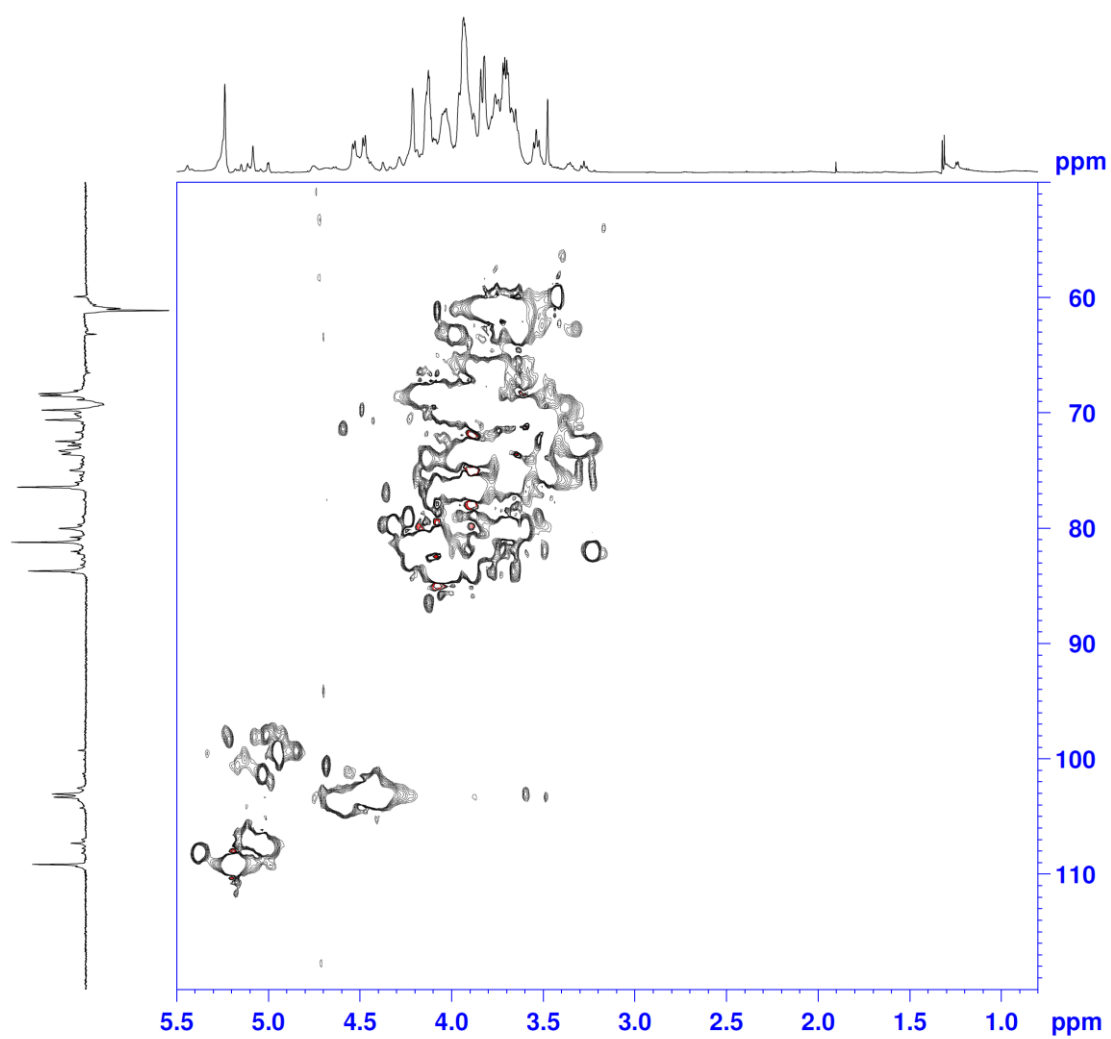


Figure S6 The HSQC spectrum of CTP3-B

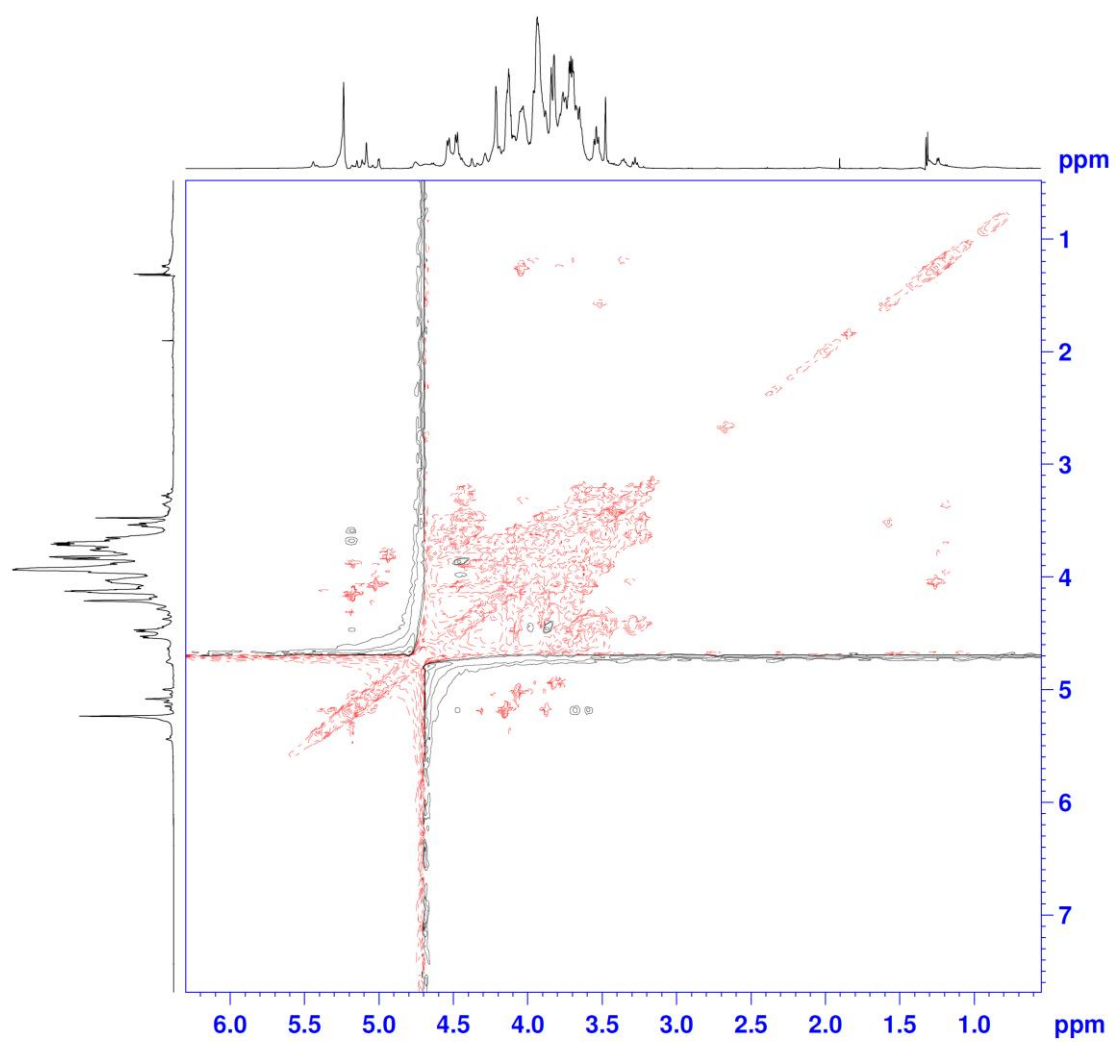


Figure S7 The TOCSY spectrum of CTP3-B

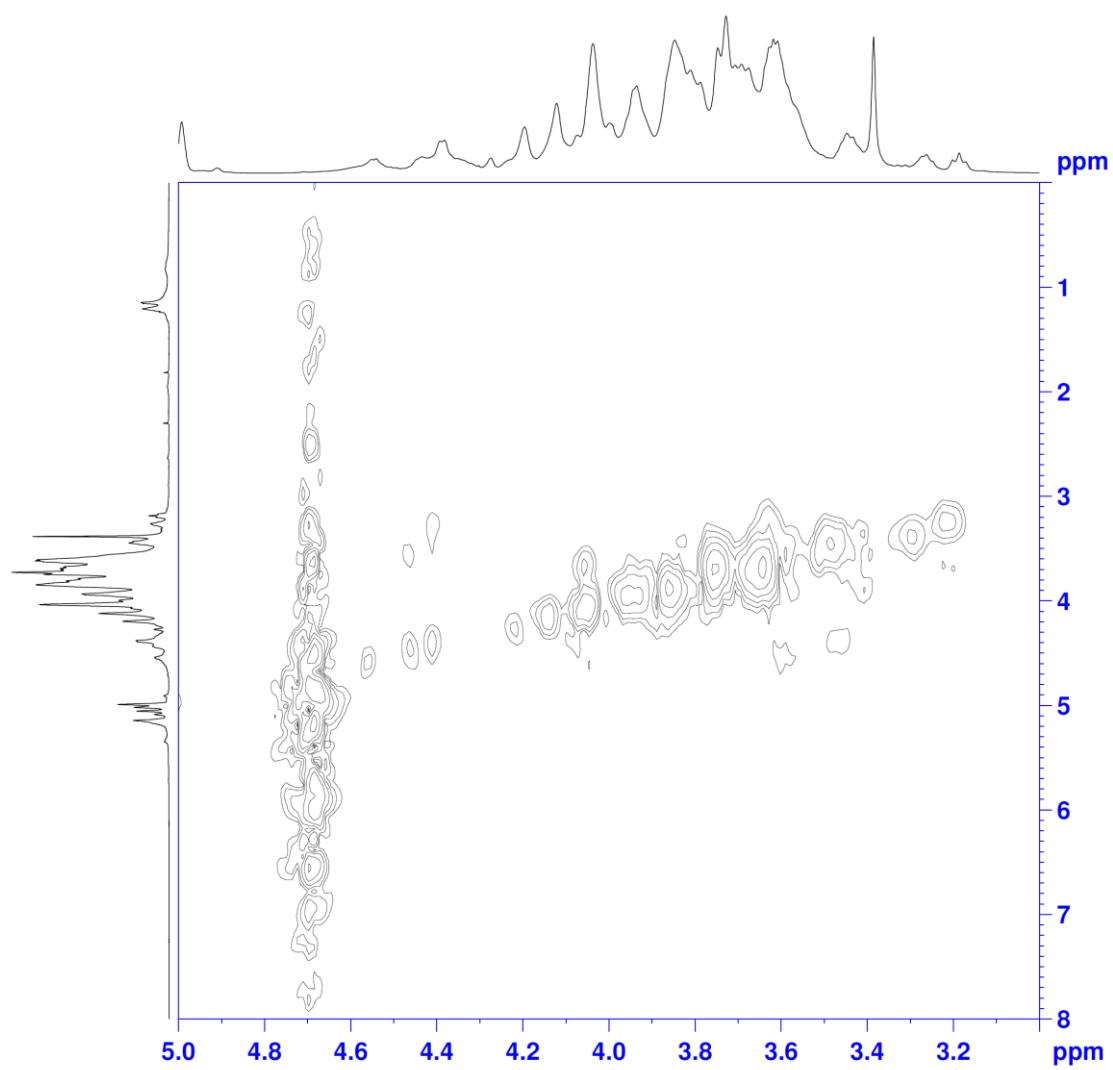


Figure S8 The COSY spectrum of CTP3-C

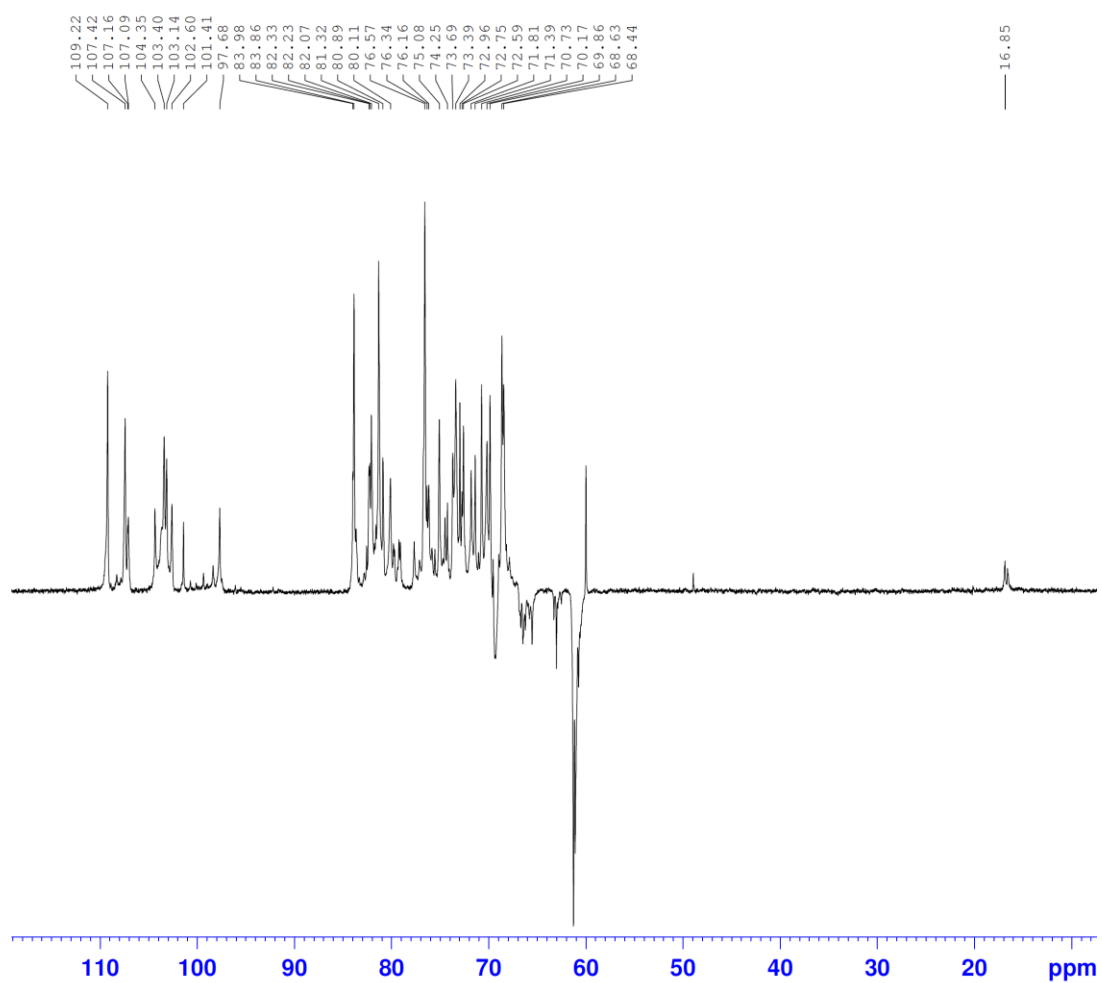


Figure S9 The DEPT spectrum of CTP3-C

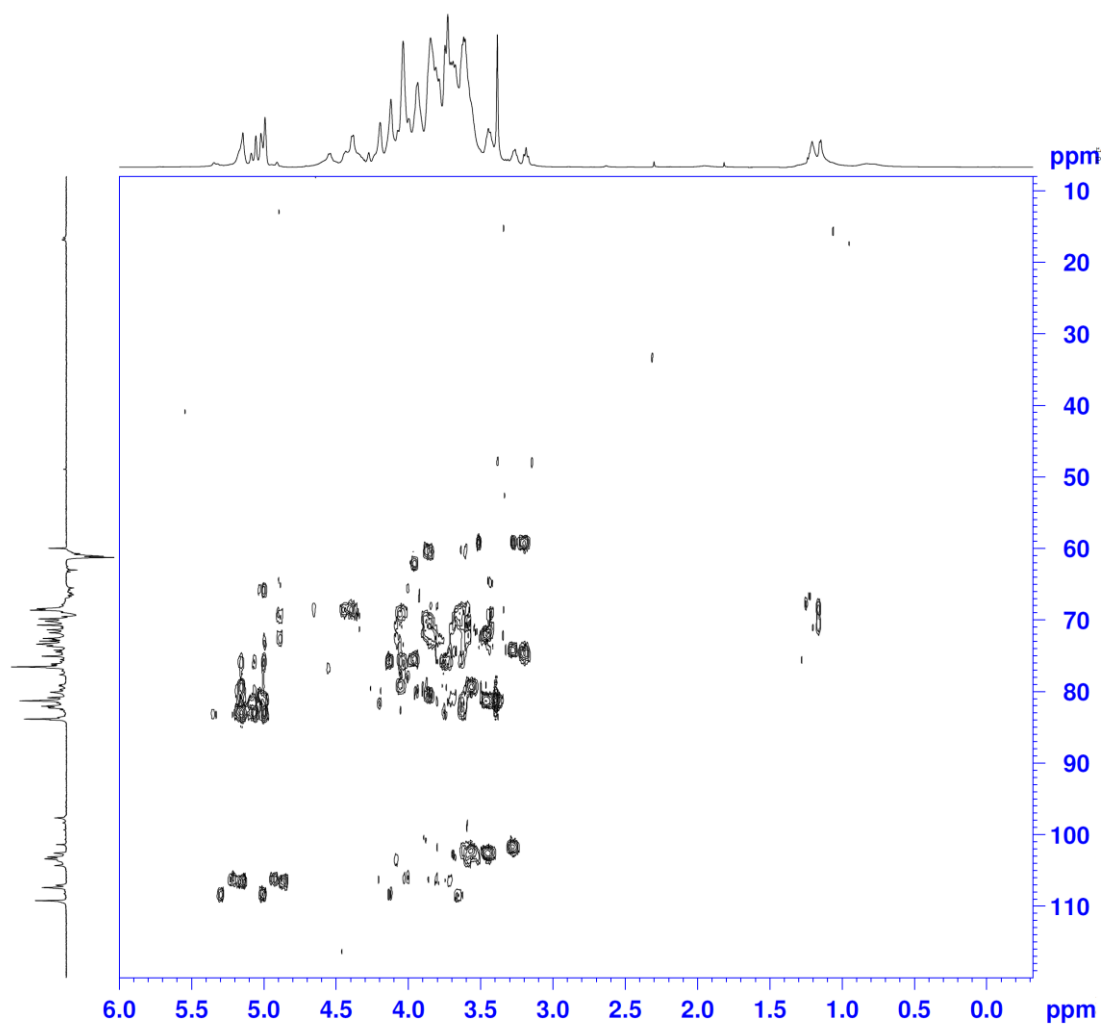


Figure S10 The HMBC spectrum of CTP3-C



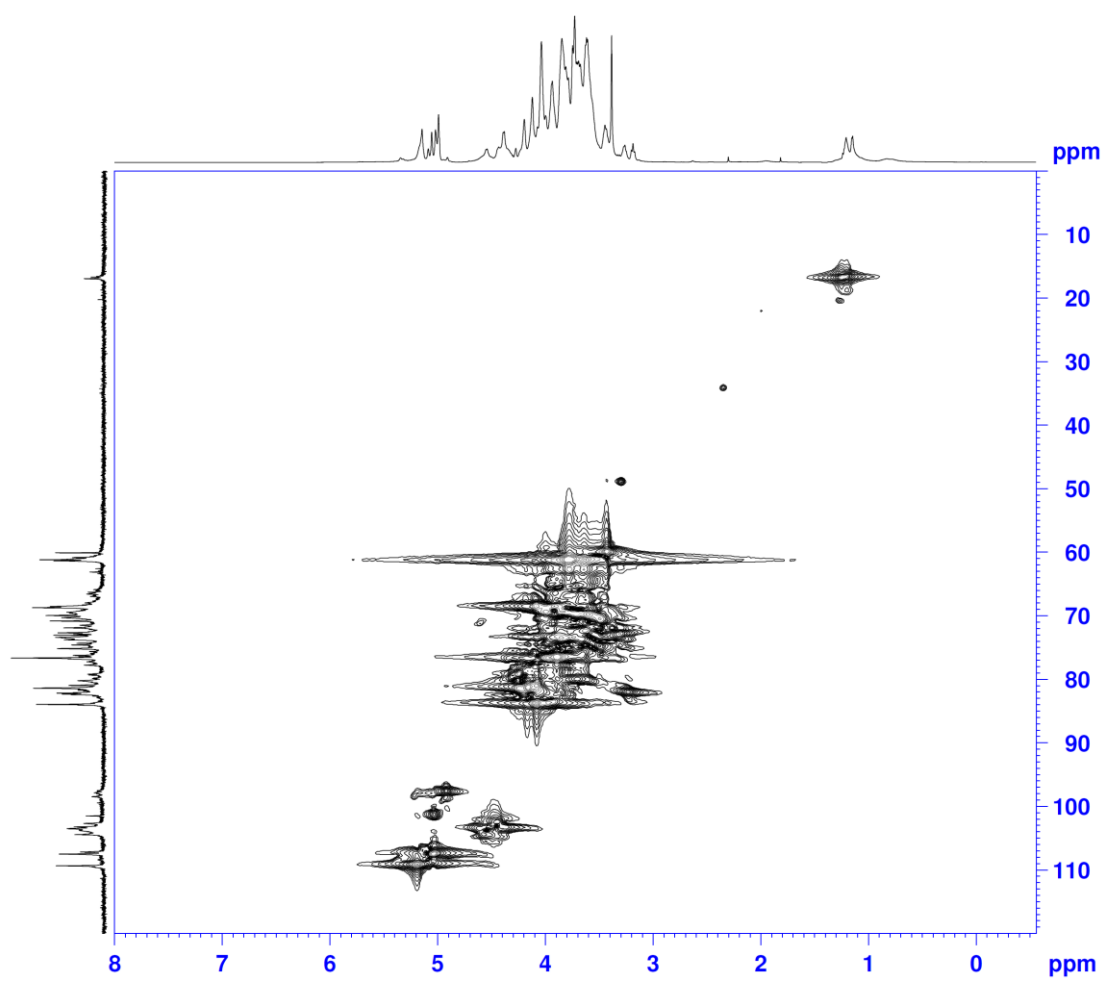


Figure S11 The HMQC spectrum of CTP3-C

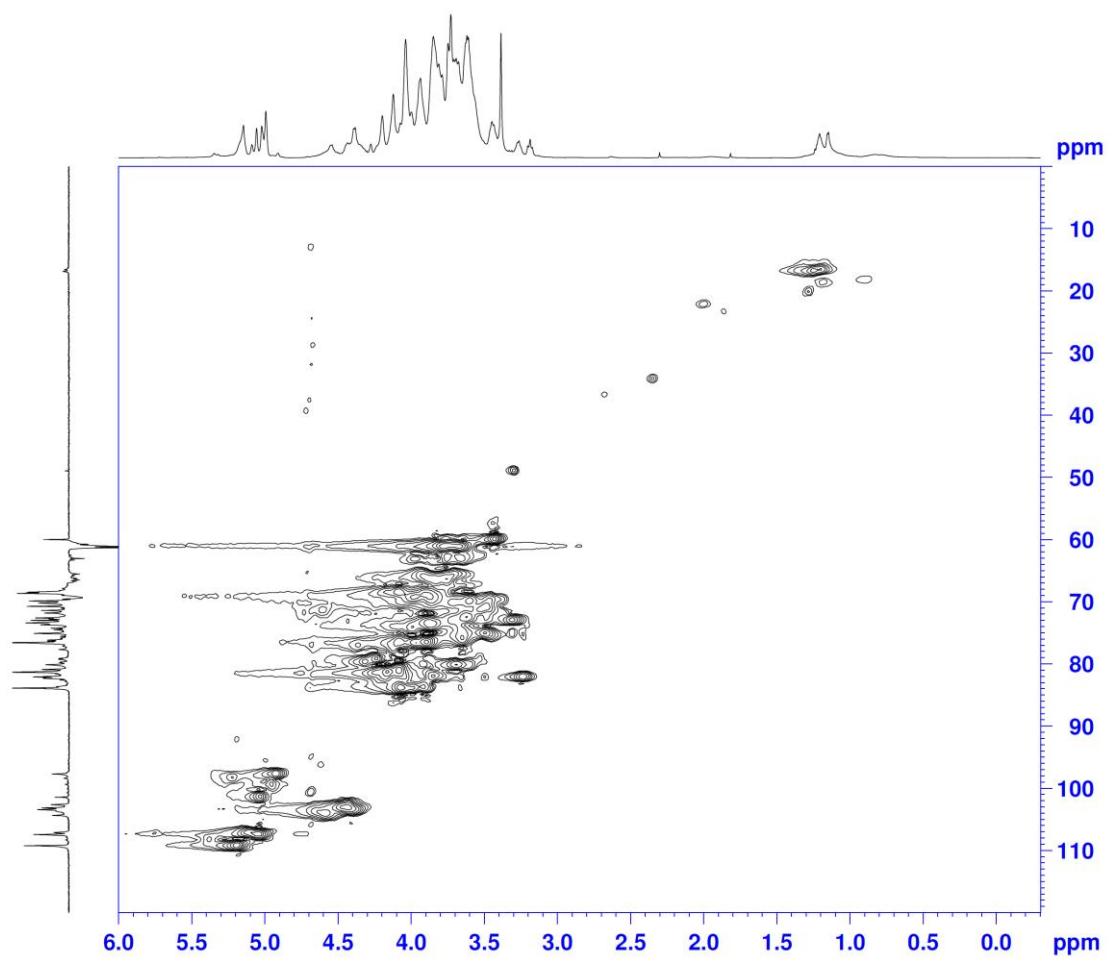


Figure S12 The HSQC spectrum of CTP3-C

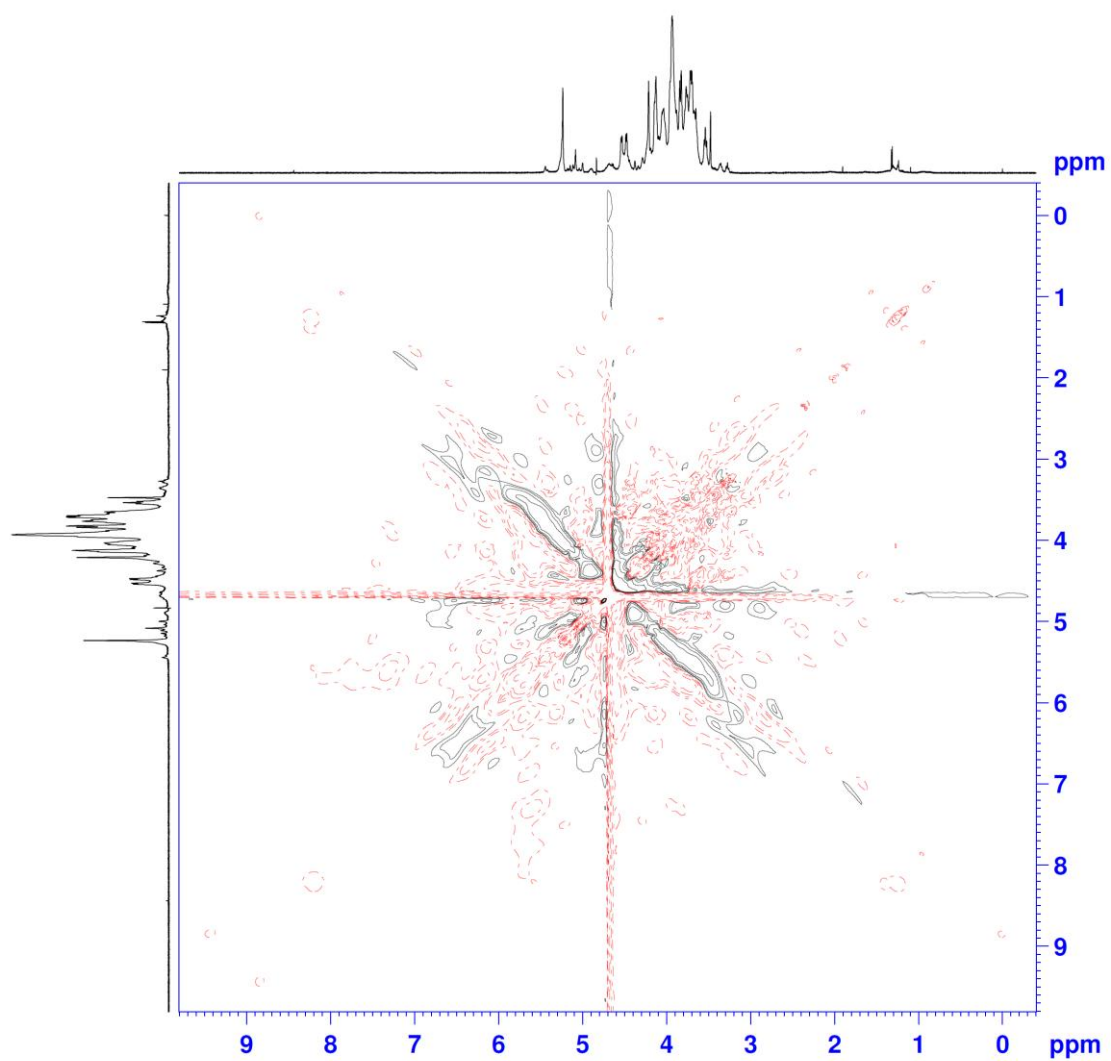


Figure S13 The TOCSY spectrum of CTP3-C

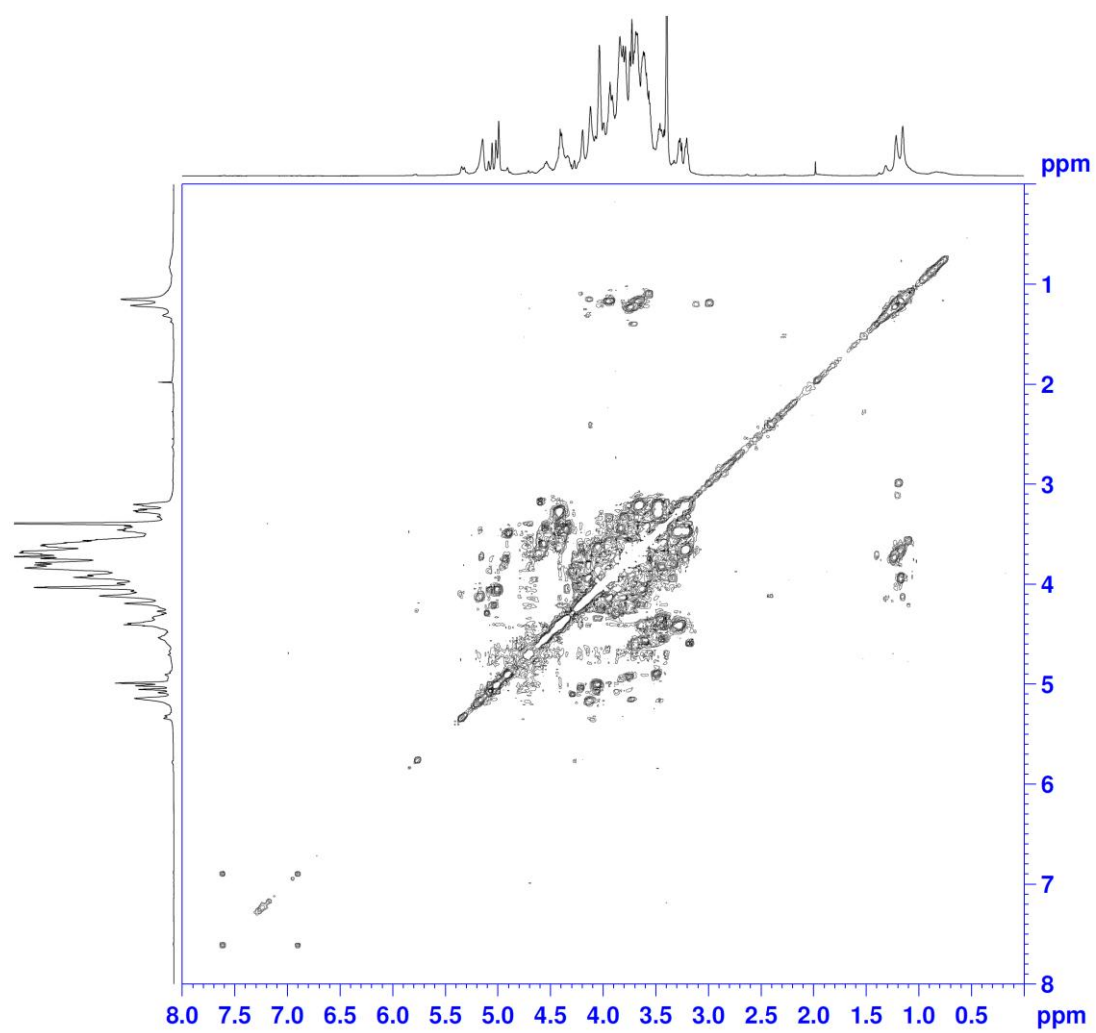


Figure S14 The COSY spectrum of CTP3-D

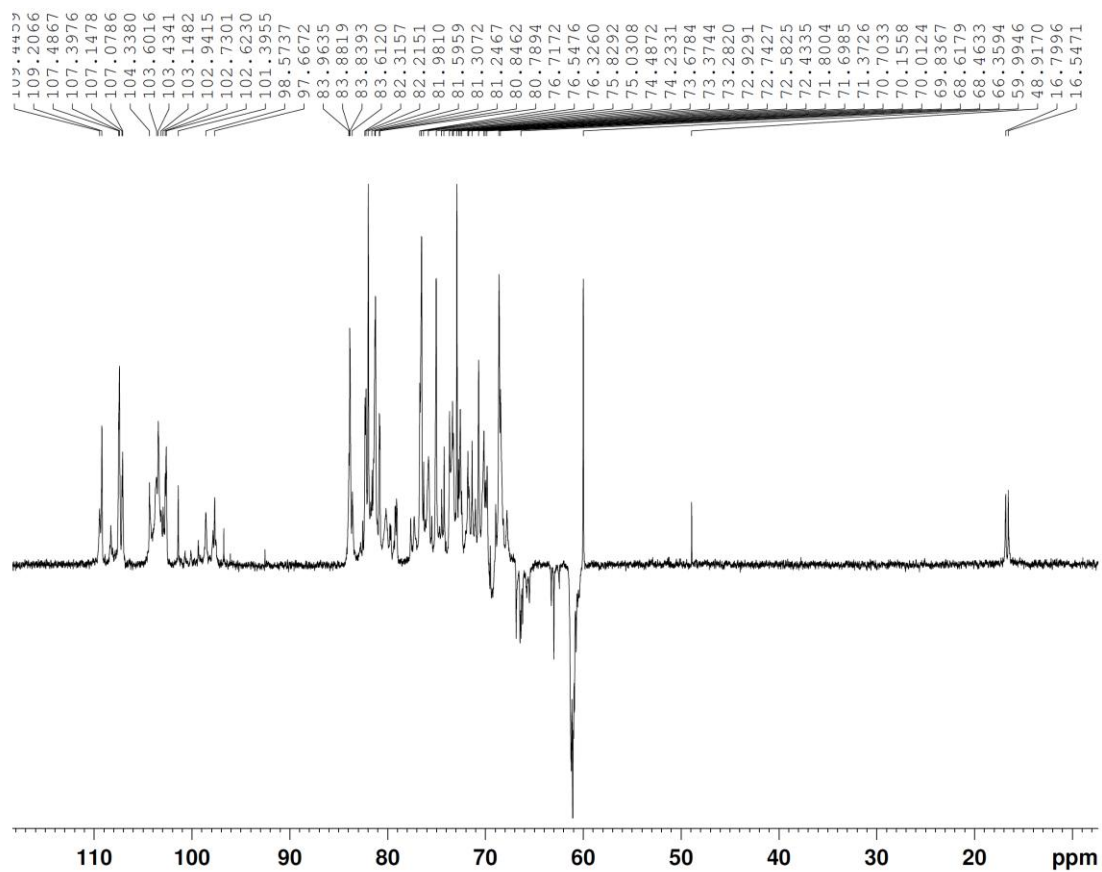


Figure S15 The DEPT spectrum of CTP3-D

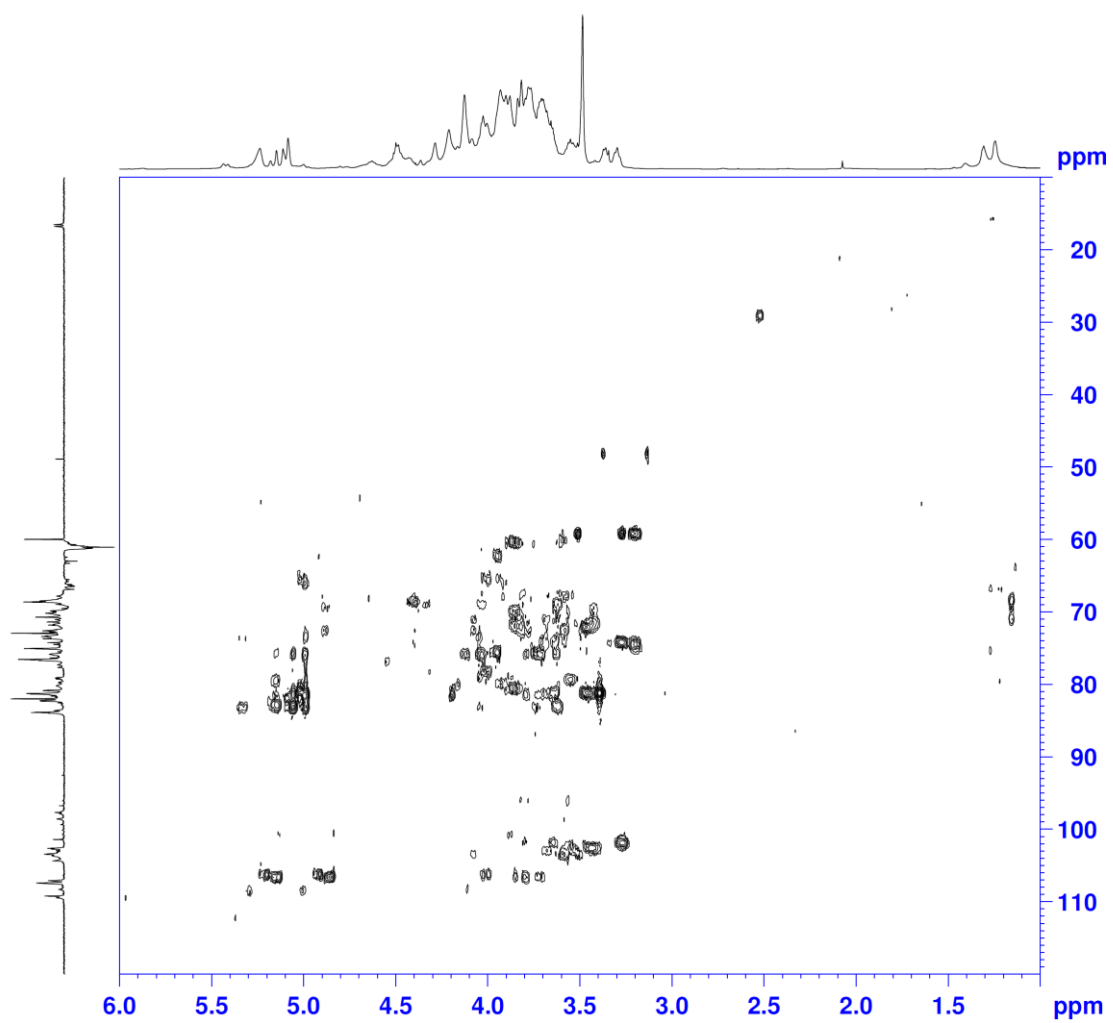


Figure S16 The HMBC spectrum of CTP3-D

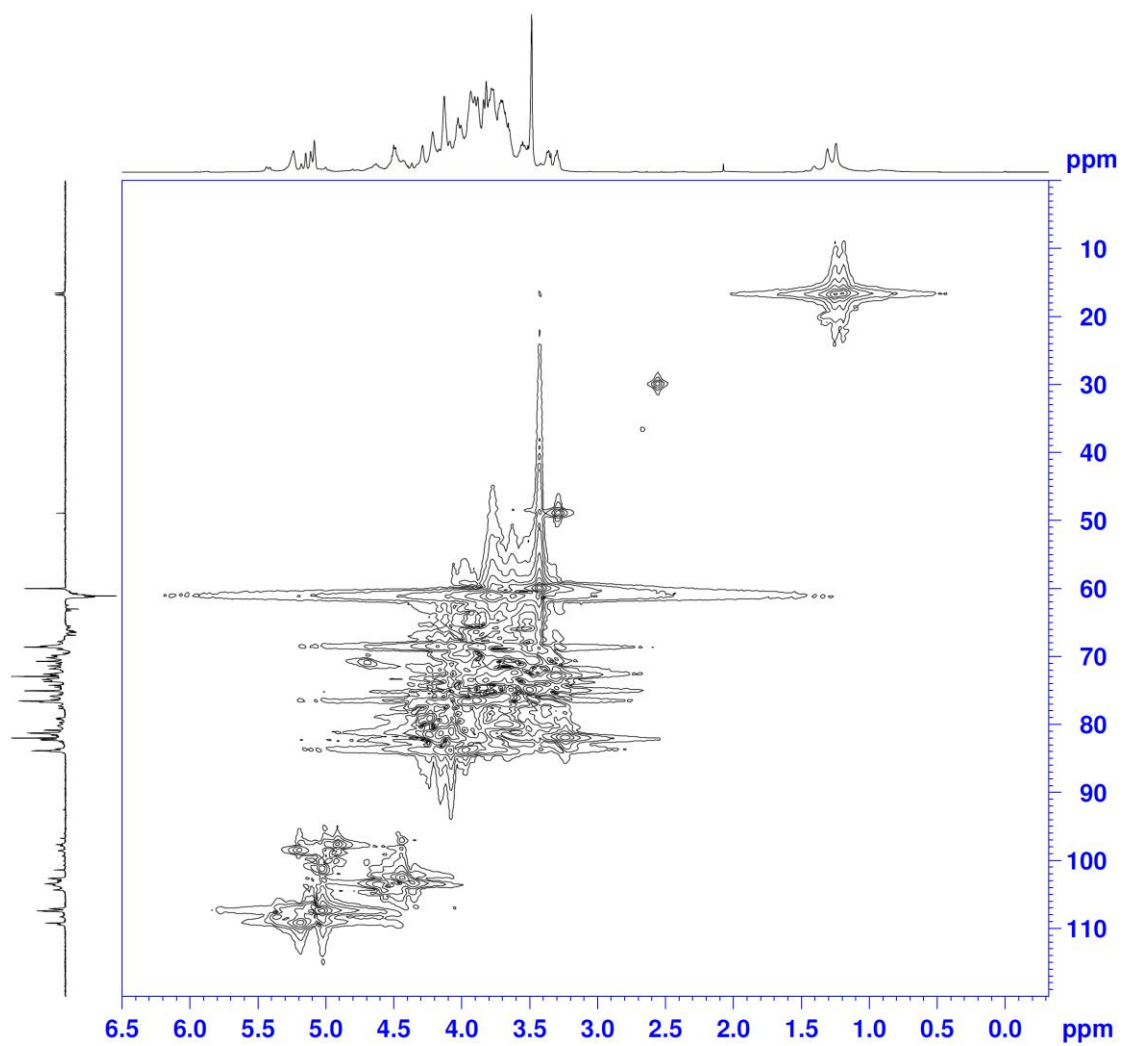


Figure S17 The HMQC spectrum of CTP3-D

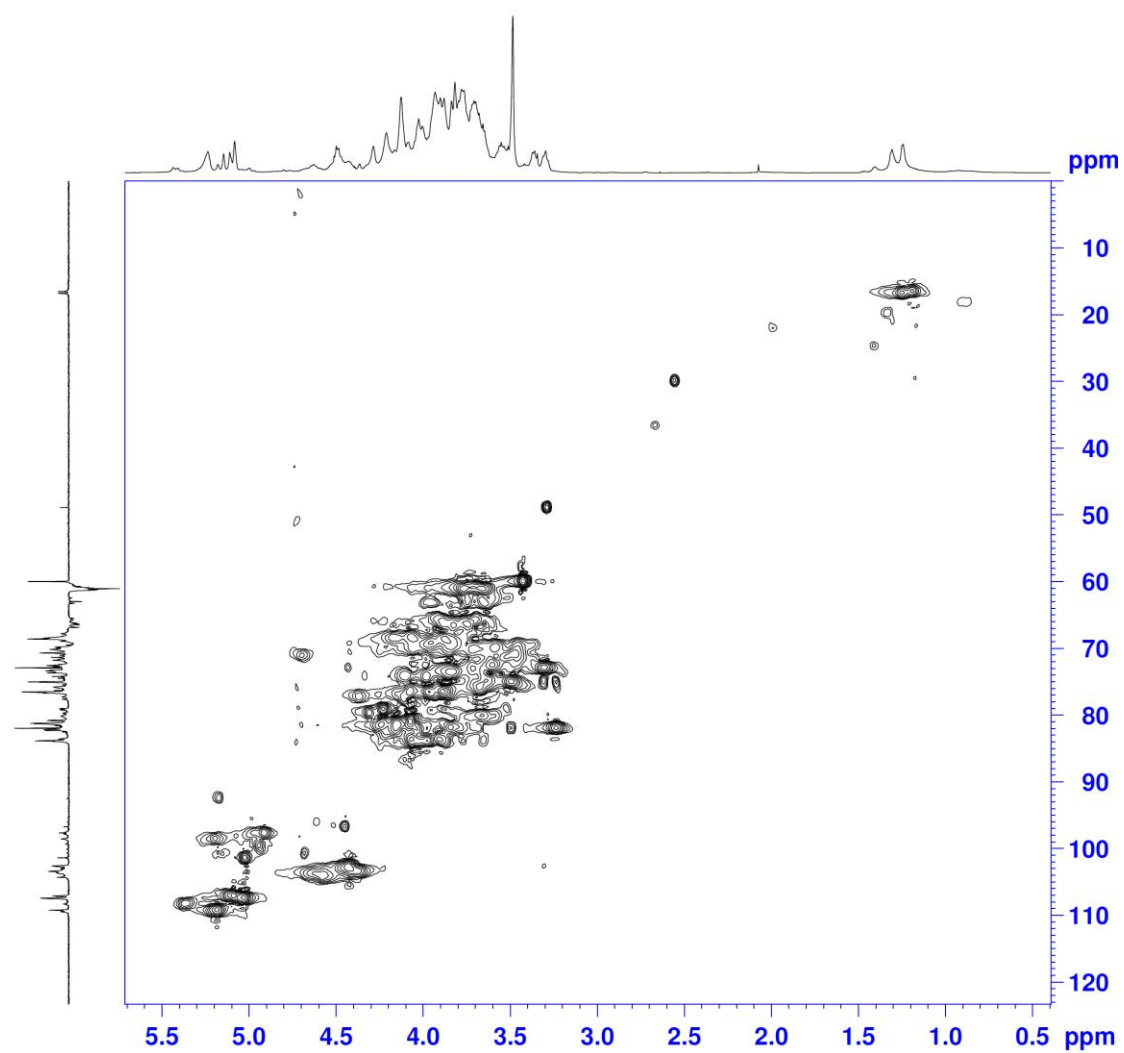


Figure S18 The HSQC spectrum of CTP3-D



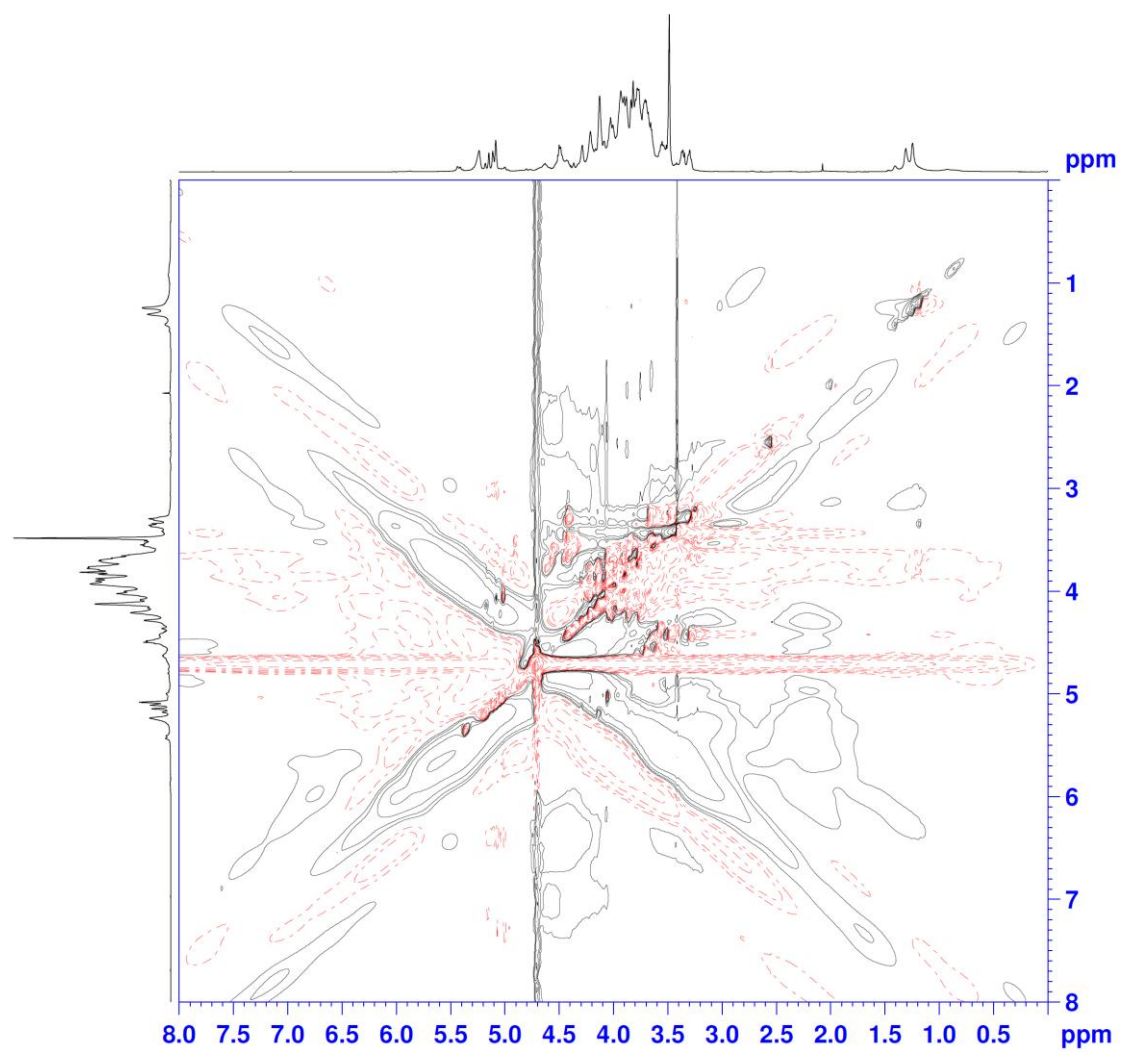


Figure S19 The TOCSY spectrum of CTP3-D

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